ISSN 1064-2293, Eurasian Soil Science, 2024, Vol. 57, Suppl. 1, pp. S57–S67. © Pleiades Publishing, Ltd., 2024.

SOIL BIOLOGY

Moisture and Temperature as Factors of the Formation and Functioning of Prokaryotic Complexes in Oil-Contaminated Soils

N. A. Manucharova[a], *, E. A. Ovchinnikova[a], **, M. A. Kovalenko[a], M. G. Alexeeva[b], A. V. Smagin[a], M. S. Rozanova[a], K. V. Pavlov[a], T. N. Nazina[c], A. P. Ershov[c], and A. L. Stepanov[a]

[a] *Lomonosov Moscow State University, Moscow, 119991 Russia*

[b] *Federal Research Center V.V. Dokuchaev Soil Science Institute, Moscow, 119017 Russia*

[c] *Winogradsky Institute of Microbiology, Research Centre of Biotechnology, Russian Academy of Sciences, Moscow, 119071 Russia*

**e-mail: manucharova@mail.ru*

***e-mail: liza.ovchinnikoff@gmail.com*

Received June 9, 2024; revised July 26, 2024; accepted July 30, 2024

Abstract—The structure of prokaryotic complexes in microcosms of oil-contaminated and control soil samples (chernozem, Voronezh oblast, gray forest soil, Tula oblast, and chestnut soil, Volgograd oblast) was studied under conditions of different matric potentials of soil water and soil temperatures using molecular biology methods (RT-PCR, metabarcoding, FISH). An increase in the content of functional genes responsible for the synthesis of catechol-2,3-dioxygenase (*xylE*) and alkane monooxygenase (*alkB*) marking the initial stage of hydrocarbon degradation was observed in the studied oil-contaminated soils against the background of a decrease in the biomass and diversity of bacteria relative to the control. It was found that an increase in the matric potential of soil water from −800 to −0.1 kPa, simultaneously with an increase in the number of metabolically active representatives of bacteria and the number of *alkB* gene copies in the oil-contaminated soils incubated at 28°C corresponded to a decrease in the residual content of alkanes to 51%. The maximum content of the *alkB* gene was observed at the maximum water holding capacity and a medium incubation temperature (28°C). The highest number of copies of the *xylE* gene was found at medium values of the matric potential of soil water and temperature. The most resistant to oil pollution genera of bacteria were determined for every studied value of the matric potential of soil water and temperature. Representatives of *Pseudomonadota* became dominant under the conditions of low temperature and high water content, and spore-forming Actinomycetota and Bacillota became dominant at increased temperature and decreased water content. The obtained results on the abundance of metabolically active prokaryotic complex of soils and its taxonomic diversity and biotechnological potential can be useful for the development of efficient strategies for bioremediation of oil-contaminated areas.

Keywords: prokaryotes-hydrocarbon destructors, Calcic Luvic Chernozem Loamic Pachic, Luvisol Loamic Cutanic, Greyzemic Calcic Someric Kastanozem Loamic, functional genes, alkanmonooxygenases, catecholdioxygenases

DOI: 10.1134/S1064229324602609

INTRODUCTION

At present, oil and oil products remain among the main pollutants of the environment [30]. In oil-contaminated soils, a decrease in the contents of humus and nutrients and an increase in soil toxicity due to the accumulation of phytotoxins are observed, and changes in the acid–base properties of the soils can lead to post-technogenic salinization [15]. An advanced method for eliminating soil contamination with oil and oil products and restoring soil ecosystems is bioremediation and, particularly, biostimulation based on the creation of optimal conditions for hydrocarbon-oxidizing activity of autochthonous microorganisms [1, 4]. The advantages of bioremediation are the small amount of waste products, minimal disturbance of the environment, relatively low cost, and virtually absent contact between humans and pollutants in the course of remediation [14]. A microbial inoculate isolated from the local environment and grown in laboratory can increase the rate of bioremediation of contaminated soil immediately after an oil spill [25]. The intensity of microbial destruction of oil hydrocarbons varies in biogeocenoses of different climatic zones, though most studies of the microbial destruction of oil compounds have been conducted under some average values of environmental factors. Such indicators as soil water content and temperature determine the rates of chemical reactions, enzyme activi-

ties, viability of cell contents, stability of genetic material, etc. Insufficient water content in the soil limits the development of microorganisms and transport and diffusion of soluble nutrients, whereas excessive moistening retards oxygen supply to microorganisms and negatively affects the rate of aerobic destruction of oil compounds. It is generally accepted that optimum conditions for bioremediation are formed in soils containing the maximum amount of water, which, at the same time, does not interfere with the diffusion of oxygen [17]. Temperature affects the chemical composition of oil and the availability of hydrocarbons to microorganisms, and this determines the efficiency of purification of contaminated sites [8, 11, 23, 24]. The role of psychrophilic and psychrotrophic microorganisms is particularly important in the areas with cold and temperate climate under conditions of low temperatures. At the same time, the maximum activity of most enzymes is known to be in the temperature range of 40–55°C, which attracts interest to the problem of oil destruction by the soil microbial complex in the zone of high temperatures [21].

Oxidation of hydrocarbons by microorganisms is the leading factor in the natural process of oil degradation. A comprehensive study of metabolically active prokaryotic complex of soils and its taxonomic diversity and biotechnological capacity under different values of the matric potential of soil water and temperature is necessary to develop efficient strategies of bioremediation.

The aim of our work was to study the taxonomic and functional structures of prokaryotic complex in oil-contaminated humus horizons of soils (typical chernozem, gray forest soil, and chestnut soil) under the conditions of different matric potentials of soil water and soil temperature regimes.

OBJECTS AND METHODS

Prokaryotic communities of soil humus horizons (A) sampled in June 2023 were studied. The range of studied soils was as follows:

(1) Deep zooturbated silt loamy calcareous typical chernozem (51°01′45″ N, 40°43′39″ E), or deep saturated deeply calcareous silt loamy migrational-mycellary chernozem on carbonate loesslike loam [3], or Calcic Luvic Chernozem Loamic Pachic [19]. It was sampled under a shelterbelt with European oak (*Quercus robur*) and Norway maple (*Ácer platanoídes*);

(2) Thin silt loamy gray forest soil (53°58′26″ N, 37°09′39″ E), or thin-humus deeply gleyed saturated noncalcareous silt loamy typical gray soil on mantle loam [3], or Luvisol Loamic Cutanic [19] (pH 5.5, C_{org} 2.1%) under the dog's mercury–hazel lime forest;

(3) Thin silt loamy chestnut soil (49°05′28″ N, 44°08′48″ E), or thin-humus highly calcareous silt loamy typical chestnut soil on loesslike loam [3], or Greyzemic Calcic Someric Kastanozem Loamic [19].

Two series of experiments were carried out. In the first series, the effects of matric potential of soil water on the diversity and functions of the prokaryotic community in oil-contaminated soils were evaluated (the matric potential values corresponded to $P = -800$ kPa (wilting point), $P = -300$ kPa (medium water content), and $P = -0.1$ kPa (maximum water content) upon incubation under constant temperature 28°C). In the second series, the influence of temperature at incubation temperatures of 4, 28, and 46°C and constant matric potential ($P = -300$ kPa) was studied.

The water retention curve was determined for every soil using the centrifugation method in modification of Smagin [9].

A part of soil samples were artificially contaminated with oil to 10% of oil products in the soil mass. Uncontaminated samples incubated at the same temperatures and water content levels served as the control. The experiment was carried out for one month.

Oil from the Azov–Kuban oil-and-gas basin (Kubanskaya Ploshchad oil field, oil well C-1, extraction depth 2800–2832 m, age N12, oil density 0.835 g/cm^3 at 15°C, cetane number 51, iodine number 0.4, funnel viscosity 1.5, kinematic viscosity at 20°C 4.9 mm^2/s).

The biodiversity of the prokaryotic complex in the samples was analyzed using the method of high-throughput sequencing of the variable V3–V4 region of the 16S rRNA gene. The total DNA was extracted using standard procedures of the Power Soil DNA Isolation Kit (MO BIO, USA) following the manufacturer's instructions. Amplification of the 16S rRNA gene fragments was carried out using degenerate primers complementary to the sequences of both bacteria and archaea: PRK341F (CCTACGGGRBGCASCAG) and PRK806R (GGACTACYVGGGTATCTAAT). The obtained PCR fragments were purified on QIAquick columns according to the manufacturer's protocol. Every PCR fragment was dissolved in 50 μL of TE-buffer, and the obtained material was sufficient for further analysis. Nucleotide sequences of variable fragments of 16S ribosomal RNA genes from metagenomic DNA samples were determined using high-throughput sequencing on an Illumina Miseq whole-genome sequencing system (reading time 39 h, number of pair-endreads 8 million). After readings from both ends of DNA were obtained, a file with forward and reverse readings representing textual description of the primary structure of linear macromolecules in the form of a sequences of monomers was formed.

Sequencing data were processed using the automated QIIME 1.9.1 algorithm, including combining forward and reverse reads, removing technical sequences, filtering sequences with low reliability scores for individual nucleotides (quality less than Q20), filtering chimeric sequences, aligning reads to the 16S rRNA reference sequence, and distributing sequences by taxonomic units using the EzBioCloud online database [13].

Table 1. Sequences of primers used to determine the contents of *alkB* and *xylE* genes

Gene	Enzyme	System of primers (5'–3')	Reference
alkB	Alkane monooxygenase	f TGGCCGGCTACTCCGATGATCGGAATCTGG r CGCGTGGTGATCCGAGTGCCGCTGAAGGTG	[29]
xylE	Catechol-2,3-dioxygenase	f CCGCCGACCTGATC(A/T)(C/G)CATG r TCAGGTCA(G/T)CACGGTCA(G/T)GA	[18]

We used classification algorithm of operational taxonomic units with open reference (Open-reference OTU) with the classification threshold of 97%.

The total number of cells and the number of metabolically active cells in the samples were determined by the luminescence microscopy method using acridine orange and Cy-3 fluorochromes. The total number of prokaryotes was determined with the help of acridine orange, which reacts with the DNA of the cells. The number of metabolically active cells was determined by staining cell rRNA with specific fluorescently labeled oligonucleotide probes, and this allowed us not only to conclude about the viability of microorganisms but also to study the microbial diversity in situ. A range of probes specific for the domains of Archaea and Bacteria were applied to the studied samples [6].

An Axioskop 2 plus (Zeiss, Germany) microscope was used for direct counting of microorganisms. The number of microbial cells contained in 1 g of soil sample was calculated according to the formula:

$$N = S_1 an / vS^2 c,$$

where N is the number of cells (or the length of the mycelium, μm) per 1 g of soil, S_1 is the area of specimen (μm^2), a is the number of cells (or mycelium length, μm) per one field of view (average value was calculated for all specimens), n is the dilution of soil suspension (mL), v is the volume of the droplet applied to the glass (mL), S_2 is the area of the fields of view of the microscope (μm^2), and c is the weighed portion of soil (g). The specific mass of microorganisms was taken to be equal to 1 g/cm^3, and the water content in the cells was taken to be equal to 80%. The biomass of microbial cells was calculated taking into account the dry biomass for one bacterial cell of 0.1 μm^3 in volume (2×10^{-14} g) and the biomass of 1 m of actinomycete mycelium of 0.5 μm in diameter (3.9×10^{-8} g) [7].

The real-time PCR method was used for quantitative analysis of the number of DNA copies of the functional genes *alkB* and *xylE* in studied variants of soil microcosms. The measurements were carried out on a DTLite4 DNA-Technology detecting amplifier. The obtained results were processed using the Real-time PCR software package. The DTLite4 detecting amplifier combines the functions of a programmable thermocycler and an optical system, which allows recording the fluorescence of the reaction mixture in test tubes during the polymerase chain reaction. The intercalating dye SYBR® Green and dyes bound with primers (Lightcyaler—two probes binding the target DNAs at a short distance from one another) were used. The 5'-end of one probe and 3'-end of second probe contain a donor fluorophore and an acceptor fluorophore. The reaction mixture was prepared from the Super MixEva Green Biorad preparation (a concentrated buffer with deoxyribonucleotides, Sso7d-fusion polymerase, MgCl2, EvaGreen dye, and stabilizers). The instrument was calibrated using the dependence of fluorescence intensity on the logarithm of DNA concentration in standard solutions. The sequences of the primers used in experiment are presented in Table 1 [18, 29]. The following temperature profiles were used:

— to amplify the *alkB* gene: 1 cycle: 94.0°C, 5 min; 30 cycles: 94.0°C, 1 min; 60.0°C, 1 min; 72.0°C, 1 min; 1 cycle: 72.0°C, 3 min [5].

— to amplify the *xylE* gene: 1 cycle: 95.0°C, 5 min; 35 cycles: 94.0°C, 1 min; 61.5°C, 1 min; 72.0°C, 2 min; 1 cycle: 72.0°C, 10 min.

The amount of studied DNA was expressed in absolute or relative units. Every quantitative determination of DNA template requires three standards and a negative control (the sample without DNA template).

The statistical analysis of the data was carried out using Statistica 8.0 software. All soil samples were analyzed in fivefold.

The content of individual hydrocarbons in soil samples was determined using gas chromatography, Weighed portion of soil (5 g) was resuspended in 10 mL of *n*-hexane, then thoroughly mixed and left for 10 min at room temperature for complete extraction. Then, 8 mL of the non-polar fraction with the dissolved oil components was taken and placed into a separation column with 2 g of silica gel sorbent. Hexane was used as an eluent, mobile phase of the column. After passing through the separation column, the sample with aliphatic fraction was evaporated to a volume of 50 μL and then analyzed by gas chromatography, Gas chromatography of oil alkanes was carried out on a Kristall 5000.1 gas chromatograph (Khromatek

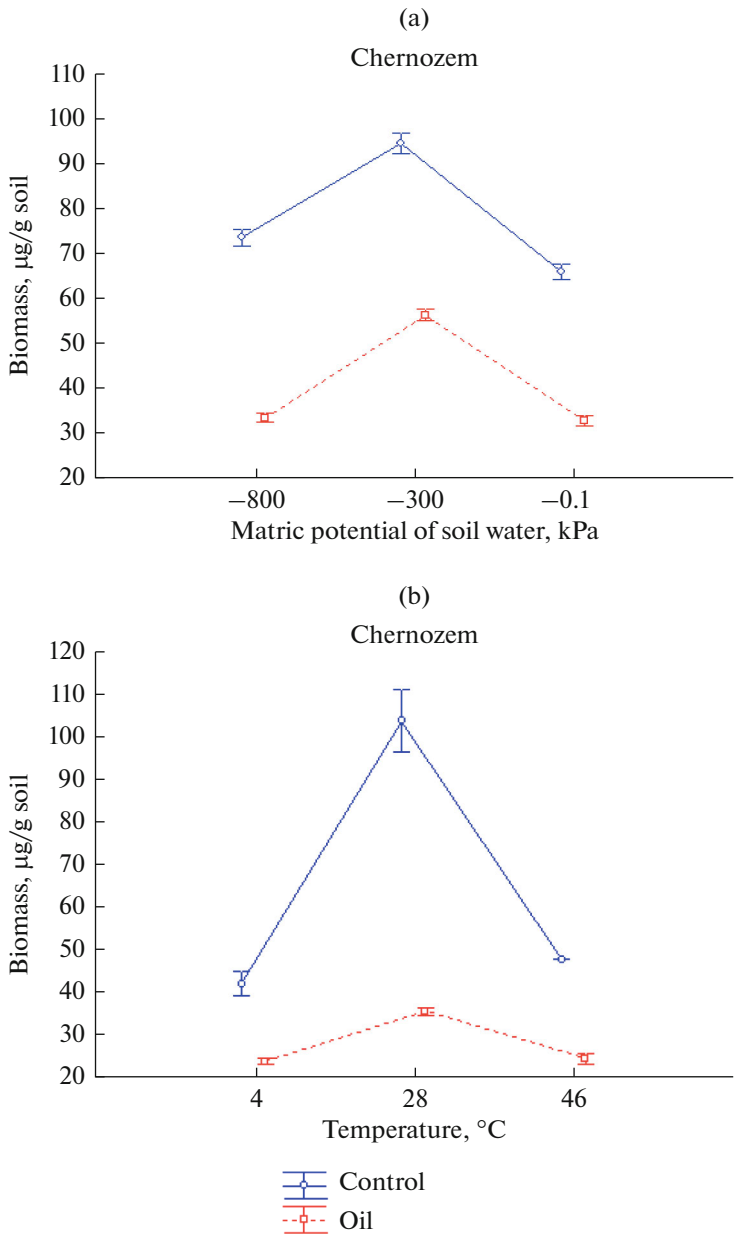

Fig. 1. Biomass of prokaryotes in the oil-contaminated chernozem and in the control under different (a) matric potentials of soil water (current effect: $F(2, 12) = 2.4376$, $p = 0.12930$; type III decomposition; vertical bars denote ± standard error) and (b) temperatures (current effect: $F(2, 12) = 34.685$, $p = 0.00001$; type III decomposition; vertical bars denote ± standard error) on the 30th day of the experiment.

Company, Russia), which had a capillary column ZB-1 with operating temperatures from 100 to 310°C and a flame ionization detector with a temperature of 320°C. The 1-μL samples were taken from the alkane fractions of oil obtained by the above described procedure.

Helium was used as a carrier gas. The content of alkanes in oil subjected to microbial degradation was measured relative to the control sample. The residual content of *n*-alkanes was calculated with the internal normalization method for phytane on the basis of the obtained chromatograms relative to the control [10].

RESULTS AND DISCUSSION

The luminescence microscopy method demonstrated that the total number and biomass of prokaryotes, as well as metabolically active representatives of prokaryotic complex, in the oil-contaminated soil were two to three times lower than those in the control

samples. This regularity was observed for all studied values of the matric potential of soil water (Fig. 1a) and for all studied temperatures (Fig. 1b).

An inverse relationship was observed between the presence of oil in the soil samples and the number of prokaryotic cells (correlation coefficient –1). The most significant decrease in the number of prokaryotic cells in contaminated soils in comparison with the control was found under the soil water content close to the wilting point at $P = -800$ kPa. The fraction of metabolically active cells in the samples of oil-contaminated soils did not exceed 30% of the total number of prokaryotic cells under all studied values of matric potential of soil water and all temperatures. The maximum number of metabolically active cells in contaminated soils was observed in the gray forest soil at $P = 0$ kPa (4.86×10^8 cells/g soil, or, in terms of biomass, 9.71 μg/g soil), and in the chernozem and chestnut soil, under the medium water content ($P = -300$ kPa (4.61×10^8 cells/g soil, or 9.22 μg/g soil and 3.83×10^8 cells/g soil, or 7.67 μg/g soil, respectively). The least significant suppression of metabolically active bacterial component in oil-contaminated soil was observed at the maximum soil water content.

The prokaryotic genome contains plasmid genes that play an adaptive role increasing the ability of microorganisms to adapt to the environmental changes. These genes are located in plasmids and are subjected to the high variability and horizontal transfer. The examples are the genes responsible for biodegradation processes, pathogenic activity, destruction of oil hydrocarbons, etc. These characteristics can be useful for microorganisms, but their presence is not necessary from the point of view of the metabolism functioning. The class of integrated membrane-associated nonhemal iron-containing monooxygenases of the *alkB* type allows a wide range of proteobacteria and actinomycetes to grow on *n*-alkanes with 5 to 16 carbon atoms [27, 28]. Catechol-2,3-dioxygenase is encoded by the *xylE* gene and catalyzes the reactions of extradiol degradation of aromatic compounds, such as benzene, toluene, xylene, phenol, naphthalene, and biphenyl via the meta pathway [12]. Compared to catechol-1,2-dioxygenases, catechol-2,3-dioxygenases are considered to be more versatile and are used in most catabolic pathways [16, 26].

Functional genes *alkB* and *xylE* encoding the synthesis of the alkane monooxygenase and catechol dioxygenase enzymes have been detected in the studies samples. The *alkB* gene encodes alkane monooxygenase, which is the key enzyme of the alkane hydroxylase system. The use of statistical methods made it possible to assess the influence of different parameters (presence of oil hydrocarbons and different values of soil water and temperature) on the content and expression of functional genes encoding the synthesis of enzymes responsible for the destruction of aliphatic and aromatic compounds of oil.

The analysis of variance showed that, against the background decrease in the total biomass of prokaryotes in the oil-contaminated soils in comparison with the control, a reliable increase in the number of metabolically active cells expressing the functional gene *alkB,* as well as in the number of copies of the genes *alkB* and *xylE,* takes place at all the studied temperatures and soil water contents (Figs. 2, 3).

The number of copies of the *alkB* gene encoding the synthesis of alkane monooxygenase increased upon the transition from the wilting point to the maximum water-holding capacity and was maximal at the medium temperature (28°C).

The highest content of the *xylE* gene encoding synthesis of catechol-2,3-dioxygenase was recorded under medium values of moisture and temperature.

It should be noted that oil biodegradation has a direct correlation with the number of copies of functional genes and the number of cells encoding the synthesis of alkane monooxygenase. On the 30th day of the experiment, the lowest content (51%) of oil alkanes was observed in the gray forest soil at $P = 0$ kPa (Fig. 4). Note that the maximum number of metabolically active biomass and the highest number of copies of the *alkB* gene were found in the gray forest soil at the maximum water content. In the chernozem and chestnut soil, the greatest loss of oil alkanes took place at $P = -300$ kPa and a temperature of 28°C. The share of *n*-alkanes relative to the control decreased in these soils to 79 and 75%, respectively. The least favorable conditions for oil biodegradation in the studied soils were formed at $P = -800$ kPa.

The method of high-throughput sequencing allowed studying the influence of moisture and temperature on the phylogenetic structure of the bacterial complex of soils. It is important to note that oil contamination resulted in a decrease in α-diversity similar to the decrease in the total number of prokaryotic cells in comparison with the control, and this agrees with earlier studies [2, 22].

At each studied temperature and moisture values, the most resistant to oil contamination bacteria of different taxonomic levels were determined. The addition of oil into the sample causes the decrease in bacterial diversity and increases the share of the dominant phylum in the community. In soils with the water content corresponding to the total water-holding capacity at the low temperature (4°C), the phylum *Pseudomonadota* predominated, and its share in the community increased in the oil-contaminated soils (Fig. 5).

It is known that some strains of the species *Pseudomonas pickettii* and *P. fluorescens* can perform biodegradation of benzene, toluene, ethyl benzene, and xylene with the participation of catechol-2,3-dioxygenase and nitrate as an alternative electron acceptor under conditions of limited oxygen access in oil-contaminated aquifers [20]. It is reported that oil-con-

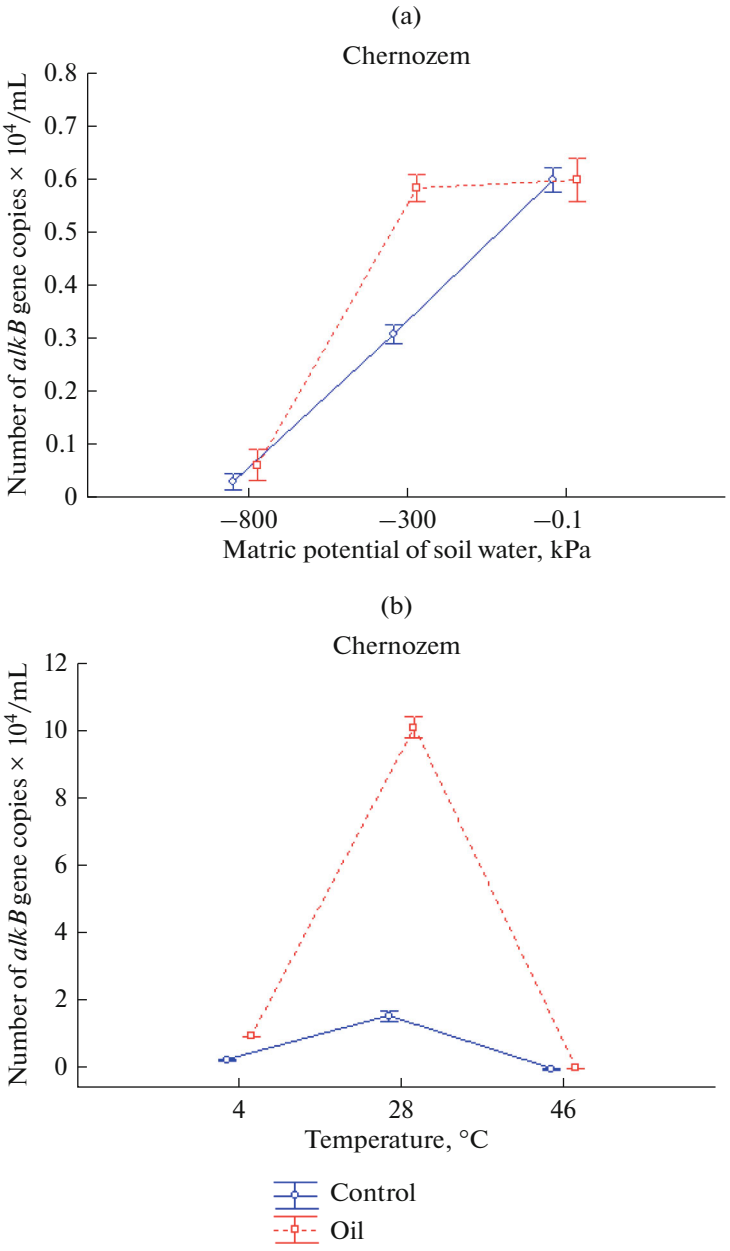

Fig. 2. Number of the *alkB* gene copies in the oil-contaminated chernozem and in the control under different (a) matric potentials of soil water (current effect: F(2, 12) = 16.112, $p = 0.00040$; type III decomposition; vertical bars denote ± standard error) and (b) temperatures (current effect: F(2, 12) = 600.92, $p = 0.00000$; type III decomposition; vertical bars denote ± standard error) on the 30th day of the experiment.

taminated groundwater with insufficient oxygen content is characterized by an increased content of genes encoding synthesis of catechol-2,3-dioxygenases.

The phylum *Actinomycetota* becomes the dominant phylum representing more than a half of the community in the soils incubated at 28°C and $P = -300$ kPa.

At the wilting point (−800 kPa), a significant predominance of the phylum *Actinomycetota* was observed in all soils, and the addition of oil resulted in an increase in its share by 10–25%. The increase in the share of the phylum Bacillota was observed at 46°C, and this phylum comprised more than 75% of the bacterial community in the chernozem.

The specificity of the bacterial complex of oil-contaminated soils is particularly pronounced at the genus level. Thus, at the low temperature (4°C), the most frequently encountered hydrocarbon-oxidizing bacteria represent the *Pseudomonas, Polaromonas,* and *Massilia* genera; at the maximum water content ($P = -0.1$ kPa), these are the *Variovorax* (11%) and

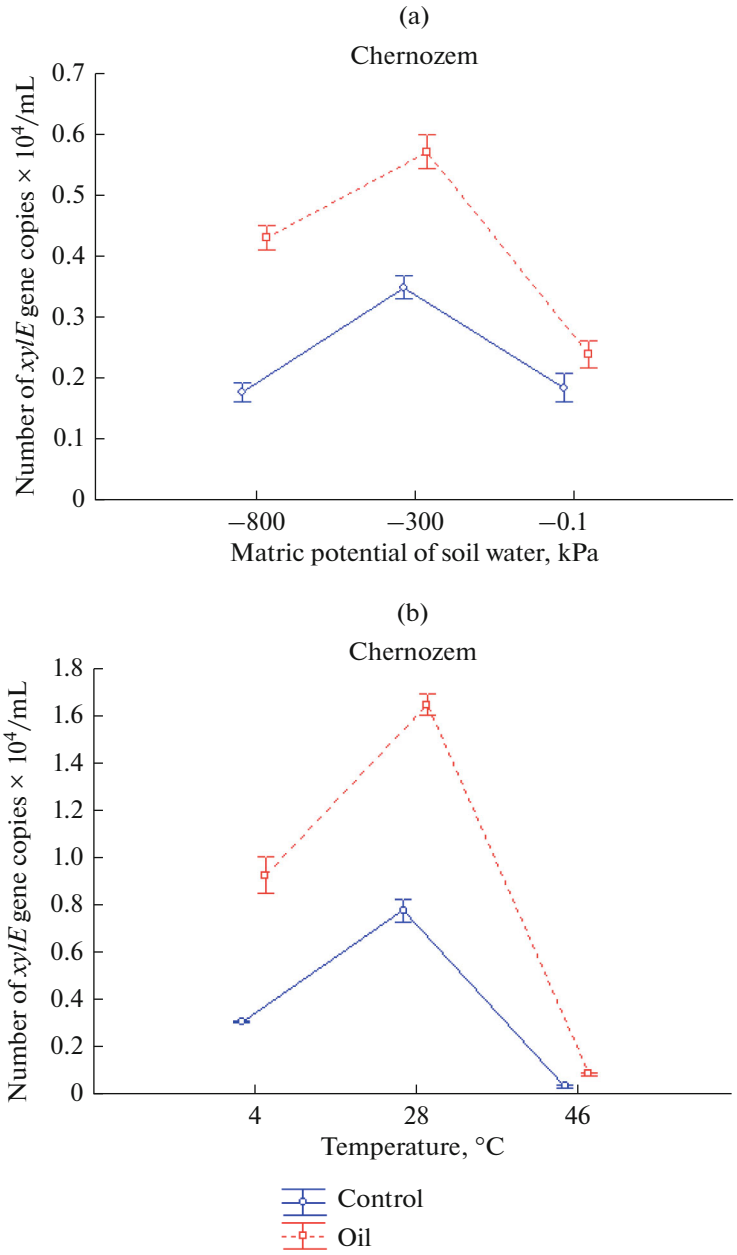

Fig. 3. Number of the *xylE* gene copies in the oil-contaminated chernozem and in the control under different (a) matric potentials of soil water (current effect: F(2, 12) = 12.320, $p = 0.00123$; type III decomposition; vertical bars denote ± standard error) and (b) temperatures (current effect: F(2, 12) = 49.246, $p = 0.00000$; type III decomposition; vertical bars denote ± standard error)) on the 30th day of experiment.

Sandaracinus (10%) genera; at the medium temperature (28°C), the *Rugosibacter, Nocardia, Streptomyces,* and *Rhodococcus* genera predominate; at the medium soil water content ($P = -300$ kPa), the *Micromonospora* and *Amycolatopsis* genera become dominant (>15% of the bacterial complex) together with the genus *Streptomyces*. At the high temperature, the dominant representatives of the phylum *Bacillota* in the chernozem are bacteria of the *Thermoactinomyces* genus; at the low water content ($P = -800$ kPa), actinobacteria of the genus *Gaiella* predominate (24% of the bacterial complex).

CONCLUSIONS

Matric potential of soil water and temperature are the control factors of the formation of prokaryotic complex in oil-contaminated soils. The decrease in

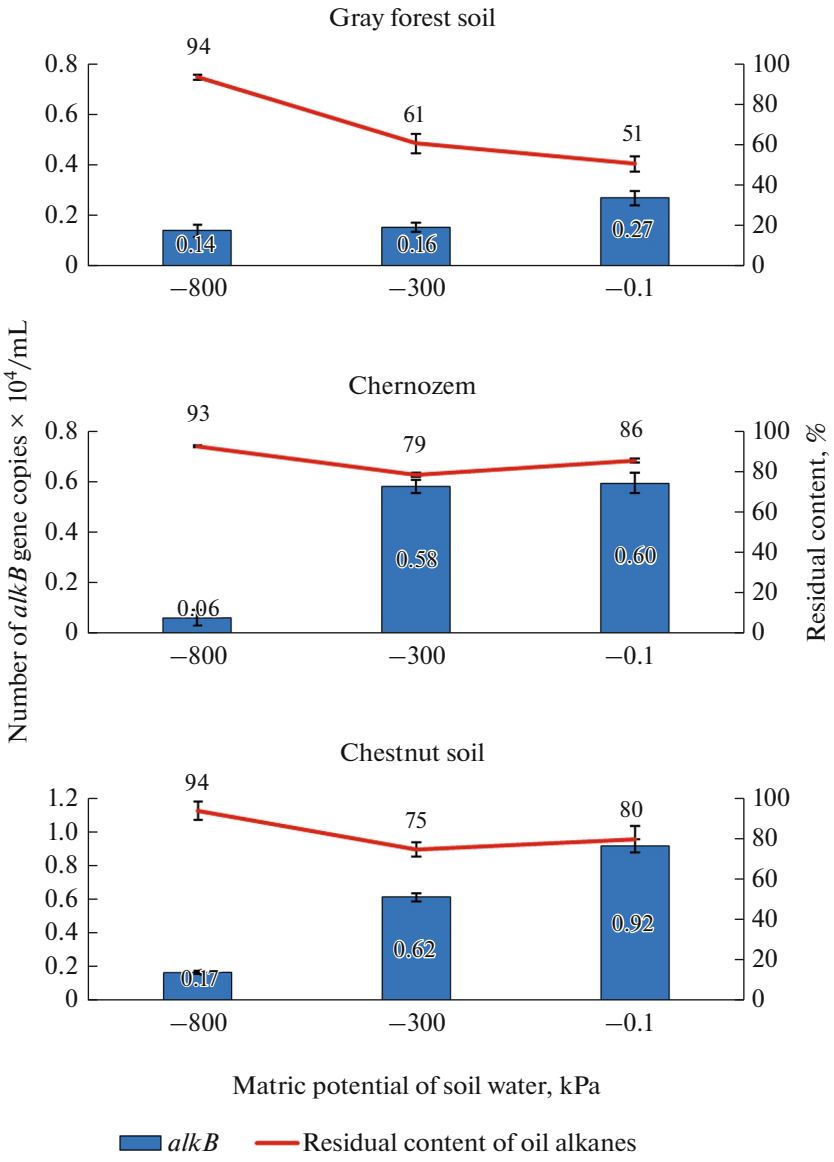

Fig. 4. Residual content of oil alkanes and relative content of *alkB* gene copies in soils under different matric potentials of soil water on the 30th day of the experiment.

the number and diversity of bacterial component in the oil-contaminated soil samples relative to the control samples is accompanied by an increase in the contents of functional genes encoding catechol-2,3-dioxygenase (*xylE*) and alkane monooxygenase (*alkB*) enzymes that participate in the biodegradation of oil compounds. An increase in the number of copies of these functional genes leads to a decrease in the content of residual oil alkanes to about 50%. An increase in the number of metabolically active representatives of bacteria and in the number of functional genes takes place with an increase in the matric pressure of soil water from −800 to −300 and −0.1 kPa at the temperature of 28°C.

The most resistant to oil contamination genera of bacteria have been determined for each studied value of the matric potential of soil water and incubation temperature. The share of the phylum *Actinomycetota* increases in all the studied soils at the low water content upon the addition of oil. At $P = -800$ kPa, the *Streptomyces* genus predominates in the gray forest and chestnut soils, and the *Gaiella* genus predominates in the chernozem. With the rise in temperature, the proportion of representatives of the phylum *Bacillota* (genus *Thermoactinomyces*) increases. With an increase in the soil water content ($P = -0.1$ kPa) and a decrease in the temperature of incubation, the *Massilia* genus, which was absent in the native soil, became the dominant in the gray forest soil; *Sandaracinus* and *Variovo-*

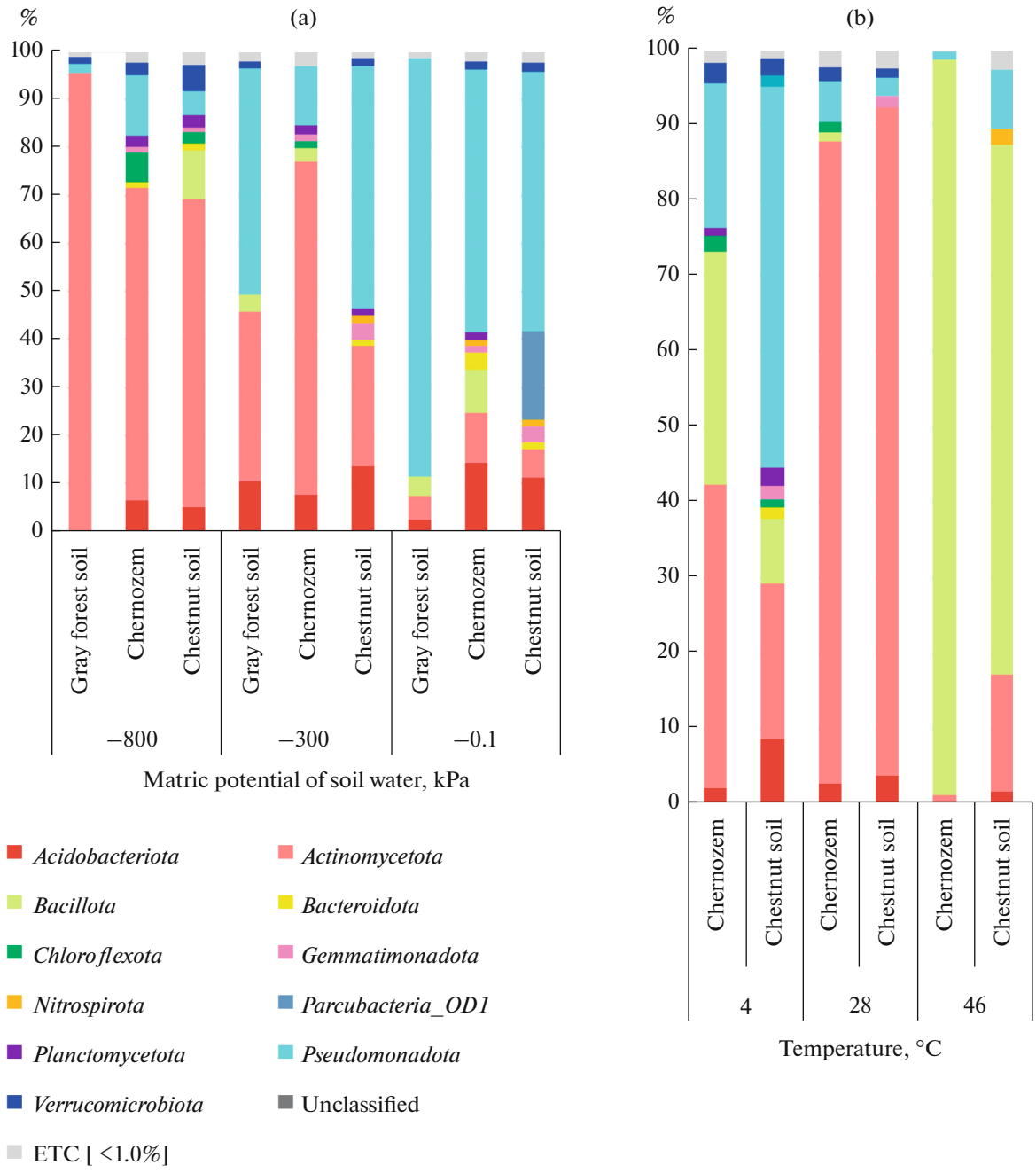

Fig. 5. Phylogenetic structure of the bacterial complex in oil-contaminated soils under different (a) matric potentials of soil water and (b) incubation temperatrures on the 30th day of the experiment.

rax genera became dominant in the chernozem; and *Phenylobacterium* and *Variovorax* genera from the phylum *Pseudomonadota* became dominant in the chestnut soil.

FUNDING

This work was supported by the Russian Science Foundation, project no. 24-14-00108 *Agrobiotechnological Potential of Autochthonous Metafunctional Soil Microbiome*.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. A. A. Vetrova, A. A. Ivanova, A. E. Filonov, V. A. Zabelin, A. B. Gafarov, S. L. Sokolov, I. A. Nechaeva, I. F. Puntus, and A. M. Boronin, "Biodegradation of oil by individual strains and principles of compiling microbial consortia for cleaning the environment from oil hydrocarbons," Izv. Tul. Gos. Univ. **2** (1), 241–257 (2013).
2. A. D. Galtsova, R. R. Kinzhaev, A. V. Arzamazova, and V. A. Romanenkov, "The effectiveness of different forms of nitrogen fertilizers in phytoremediation on oil-contaminated typical chernozem," Moscow Univ. Soil Sci. Bull. **79** (1), 28–36 (2024). https://doi.org/10.3103/S0147687424010022
3. *Classification and Diagnostics of Soils in Russia* (Oikumena, Smolensk, 2004) [in Russian].
4. S. I. Kolesnikov, K. Sh. Kazeev, T. V. Denisova, and E. V. Dadenko, "Estimation of the resistance of soils in the south of Russia to chemical pollution," Nauchn. Zh. Kuban. Gos. Agrar. Univ. **91** (7), 1–11 (2013).
5. A. V. Korshunova, T. P. Tourova, N. M. Shestakova, E. M. Mikhailova, A. B. Poltaraus, and T. N. Nazina, "Detection and transcription of *n*-alkane biodegradation genes (*alkB*) in the genome of a hydrocarbon-oxidizing bacterium *Geobacillus subterraneus* K," Microbiology (Moscow) **80** (5), 682–691 (2011). https://doi.org/10.1134/S0026261711050079
6. N. A. Manucharova, N. A. Ksenofontova, T. D. Karimov, A. P. Vlasova, G. M. Zenova, and A. L. Stepanov, "Changes in the phylogenetic structure of the metabolically active prokaryotic soil complex induced by oil pollution," Microbiology (Moscow) **89** (2), 219–230 (2020). https://doi.org/10.1134/S0026261720020083
7. L. M. Polyanskaya, V. V. Geidebrekht, A. L. Stepanov, and D. G. Zvyagintsev, "Distribution of the number and biomass of microorganisms by the profiles of zonal soil types," Pochvovedenie, No. 2, 322–328 (1995).
8. I. A. Pyrchenkova, A. B. Gafarov, I. F. Puntus, A. E. Filonov, and A. M. Boronin, "Selection and characterization of active psychrotrophic microbial oil-degrading microorganisms," Appl. Biochem. Microbiol. **42** (3), 263–269 (2006).
9. A. V. Smagin, N. B. Sadovnikova, and M. B. A. Mizuri, "Determination of the main hydrophysical characteristics of soils by centrifugation method," Pochvovedenie, No. **11**, 1362–1370 (1998).
10. *Modern Methods of Oil Studies. Reference and Methodological Manual*, Ed. by A. I. Bogomolov (Nedra, Leningrad, 1984) [in Russian].
11. R. L. Casimiro, B. Thesis (Tampere University of Applied Sciences, Tampere, 2014), p. 42.
12. C. E. Cerniglia, "Microbial metabolism of polycyclic aromatic hydrocarbons," Adv. Appl. Microbiol. **30**, 31–71 (1984). https://doi.org/10.1016/S0065-2164(08)70052-2
13. M. Chalita, Y. O. Kim, S. Park, H. S. Oh, J. H. Cho, J. Moon, N. Baek, et al., "EzBioCloud: a genome-driven database and platform for microbiome identification and discovery," Int. J. Syst. Evol. Microbiol. **74** (6), 6 (2024). https://doi.org/10.1099/ijsem.0.006421
14. I. Declercq, V. Cappuyns, and Y. Duclos, "Monitored natural attenuation (MNA) of contaminated soils: state of the art in Europe-a critical evaluation," Sci. Total Environ., No. 426, 393–405 (2012). https://doi.org/10.1016/j.scitotenv.2012.03.040
15. C. P. Devatha, A. V. J. Vishnu, and J. C. R. Purna, "Investigation of physical and chemical characteristics on soil due to crude oil contamination and its remediation," Appl. Water Sci. **9**, 89 (2019). https://doi.org/10.1007/s13201-019-0970-4
16. K. W. George and A. G. Hay, "Bacterial strategies for growth on aromatic compounds," Adv. Appl. Microbiol. **74**, 1–33 (2011). https://doi.org/10.1016/B978-0-12-387022-3.00005-7
17. A. Haghollahi, M. H. Fazaelipoor, and M. Schaffie, "The effect of soil type on the bioremediation of petroleum contaminated soils," J. Environ. Manage. **180**, 197–201 (2016). https://doi.org/10.1016/j.jenvman.2016.05.038
18. B. Hendrickx, H. Junca, J. Vosahlova, A. Lindner, I. Ruegg, M. Bucheli-Witschel, F. Faber, et al., "Alternative primer sets for PCR detection of genotypes involved in bacterial aerobic BTEX degradation: distribution of the genes in BTEX degrading isolates and in subsurface soils of a BTEX contaminated industrial site," J. Microbiol. Methods **64**, 250–265 (2006). https://doi.org/10.1016/j.mimet.2005.04.018
19. IUSS Working Group WRB, *World Reference Base for Soil Resources 2014: International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. World Soil Resources Reports No. 106* (FAO, Rome, 2014), p. 181. ISBN:978-92-5-108370-3
20. J. J. Kukor and R. H. Olsen, "Catechol 2,3-dioxygenases functional in oxygen-limited (hypoxic) environments," Appl. Environ. Microbiol. **62**, 1728–1740 (1996). https://doi.org/10.1128/aem.62.5.1728-1740.1996
21. R. R. Kumar, S. Gupta, and R. Verma, "Bioremediation of petroleum-contaminated soil. Crude oil," in *New Technologies and Recent Approaches* (2022), p. 22. https://doi.org/10.5772/intechopen.100220
22. Q. Liu, W. He, W. Zhang, L. Wang, and J. Tang, "Metagenomic analysis reveals the microbial response to petroleum contamination in oilfield soils," Sci. Total Environ. **912**, 11 (2024). https://doi.org/10.1016/j.scitotenv.2023.168972
23. B. A. Mekonnen, T. A. Aragaw, and M. B. Genet, "Bioremediation of petroleum hydrocarbon contaminated soil: a review on principles, degradation mechanisms, and advancements," Front. Environ. Sci. **12**, 10 (2024). https://doi.org/10.3389/fenvs.2024.1354422
24. R. Patowary, A. Devi, and A. K. Mukherjee, "Advanced bioremediation by an amalgamation of nanotechnology and modern artificial intelligence for efficient restoration of crude petroleum oil-contaminated sites: a prospective study," Environ. Sci. Pollut. Res., No. 30, 59–84 (2023). https://doi.org/ https://doi.org/10.1007/s11356-023-27698-4
25. B. Stallwood, J. Shears, P. A. Williams, and K. A. Hughes, "Low temperature bioremediation of oil-contaminated soil using biostimulation and bioaugmentation with a Pseudomonas sp. from maritime Antarctica," J. Appl.

Microbiol. **99** (4), 794–802 (2005). https://doi.org/10.1111/j.1365-2672.2005.02678.x

26. F. H. Vaillancourt, J. T. Bolin, and L. D. Eltis, "The ins and outs of ring-cleaving dioxygenases," Crit. Rev. Biochem. Mol. Biol. **41**, 241–267 (2006). https://doi.org/10.1080/10409230600817422
27. J. B. van Beilen, Z. Li, W. A. Duetz, T. H. M. Smits, and B. Witholt, "Diversity of alkane hydroxylase systems in the environment," Oil Gas Sci. Technol. **58** (4), 427–440 (2003). https://doi.org/10.2516/ogst:2003026
28. J. B. van Beilen and E. G. Funhoff, "Alkane hydroxylases involved in microbial alkane degradation," Appl. Microbiol. Biotechnol. **74**, 13–21 (2007). https://doi.org/10.1007/s00253-006-0748-0
29. L. G. Whyte, T. H. M. Smits, D. Labbe, B. Witholt, C. W. Greer, and J. B. van Beilen, "Gene cloning and characterization of multiple alkane hydroxylase systems in *Rhodococcus* strains Q15 and NRRL B-16531," Appl. Environ. Microbiol. **68** (12), 5933–5942 (2002). https://doi.org/10.1128/AEM.68.12.5933-5942.2002
30. B. K. Yadav and S. M. Hassanizadeh, "An overview of biodegradation of LNAPLs in coastal (semi)-arid environment," Water Air Soil Pollut. **220**, 225–239 (2011). https://doi.org/10.1007/s11270-011-0749-1

Translated by T. Chicheva